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K R ANEJA

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About the Author

Prof. (Dr.) K. R. Aneja is the former Chairman of the Department of Microbiology, Kurukshetra University, Kurukshetra and is currently an **Expert Member** of the **Project Expert Group** of the Indian Council of Forestry Research and Education (ICFRE). He has recently been selected for the **Life Time Achievement Award-2022** by the Mycological Society of India. He had served as the **Governor's/Chancellor's nominee** for Teacher's selection at Punjabi University Patiala, Punjab; **President** of the Mycological Society of India, **Recorder of Plant Sciences Section ISCA,** and **Member RPC, ICFRE,** Dehradun. He is the recipient of **INSA-Royal Society Academic Exchange Fellowship, Dr. E.J. Butler Lecture Award** by IMS; **Rashtriya Guarv, Best Citizens of India** and **ISWS Lecture Awards**. He is the elected **Fellow of five Academic societies:** IBS, IPS, IMS, MSI and SBCA. As a researcher, he has handled 6 major research projects financed by the UGC, Ministry of Environment and Forests, Govt. of India, and DFID, United Kingdom; supervised several MPhil and 23 PhD students and published 175 research papers/reviews/book chapters in peer reviewed journals/proceedings/books in different areas, including fungal taxonomy, enzymology, environmental microbiolgy (decomposition of plant residues, aerospora, mycoherbicidal control of parthenium, water hyacinth, horse purslane), food microbiolgy (microbial spoilage, biopreservatives), medical microbiolgy (antimicrobial substances, ear pathogens, dental caries, candidiasis), thermophilic molds and mushroom composting, and pharmaceutical properties of medicinal plants and synthetic compounds. Dr. Aneja has **authored 10 books, edited 3 books** and **written 2 manuals** published by International and National publishers, in addition to the Proceedings of an International Conference organized in 1997.The books/manuals published by NEW AGE International publishers include: *An Introduction to Mycology, A Textbook of Basic and Applied Microbiology, Medical Microbiology, Fundamental Agricultural Microbiolgy, Experiments in Microbiology Plant Pathology Tissue Culture and Microbial Biotechnology*, in addition to *Fungal Diversity and Biotechnology*.

SIXTH EDITION

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Preface to the Sixth Edition

Microbiology is defined as the study of microorganisms (microbes) algae, fungi, bacteria, archaea, viruses, protozoa, viroids, prions and helminths that are too small to be seen with naked eyes. The science that deals with the variety and variations of living organisms comes under the umbrella of **biodiversity**. Microorganisms are everywhere found almost all natural elements on our planet that continually recycle key nutrients and shape our day-to-day existence and are essential for keeping the planet healthy. However, some act as pathogens of plants, animals and human beings causing dangerous diseases. The study of microbial human diseases is termed **medical microbiology** and that of plants is called **plant pathology**.

Biotechnology refers to the applications of living organisms and their components to industrial products and processes. There are several ways in which microbes, plants and animals can potentially be exploited in biotechnology using microbial and tissue culture, and rDNA technology. The use of microorganisms to obtain an economically valuable product or activity at a commercial or large scale is termed as **microbial biotechnology** or **industrial microbiology** and **mycotechnology**, if fungi are used. The microorganisms used in industrial processes are natural, laboratory selected mutants or genetically engineered strains.

In the course of the preparation of this new edition, the manual reached the notable landmark of 30 years of interrupted publication: the original version *"Experiments in Microbiology, Plant Pathology* and *Biotechnology"* first appeared in 1991. As always, the manual has been thoroughly updated. I continue to be concerned about the needs of the UG/PG/Research students/Scientists of Microbiology, Biotechnology, Biochemistry, Dairy, Food and Nutrition, Botany, Plant Pathology, Agriculture, Forestry, Environment, Medical, Nursing and scientists working in the baking, brewing, food, spawning, mushroom production, environment, diagnostic and sewage treatment industries.

Keeping in view the advances made, all the 23 Sections have been updated. Six new Appendices have been added :

- Current classification of *Fungi*.
- Culture collection centers for bacteria- International and national.
- Culture collection centers for fungi-International and national.
- Culture collection centers for protozoa and algae-International and national.
- Criteria for the Identification of fungal organisms.

Hopefully, the new edition of the manual will prove to be very useful and valuable to the students/scholars/teachers of diverse areas of biological sciences and scientists working in various related industries.

Feedback

Valuable suggestions from the esteemed readers are welcome for further improvement/ additions of the manual at my mail (anejakr@gmail.com, anejakr@yahoo.ca).

KURUKSHETRA (India) **Prof. (Dr.) K.R.ANEJA**

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1

Scope and Historical Development of Microbiology

INTRODUCTION

Microbiology is the study of **microbes (microorganisms)** such as bacteria, archaea, algae, fungi, protozoa, helminths, viruses, prions and viriods that are too small and cannot be seen with naked eye. While bacteria and archaea are classed as **prokaryotes** (Gr. *pro* = before and *karyon* = nut or kernel: organisms with a primordial nucleus); the fungi, algae, and protozoa are **eukaryotes** (Gr. *eu* = true and *karyon* = nut or kernel: organisms with membrane enclosed nucleus); viruses are **acellular** (or **subcellular**) ultramicroscopic entities are classified separately. Even smaller acellular agents of disease are **viroids** (nucleic acid without a protein coating), and **prions** (infectious proteins without any nucleic acid). Viroids cause various plant diseases, whereas prions cause mad cow disease and disorders of the central nervous system called **transmissible spongiform encephalopathies (TSEs)**.

Microorganisms were the first living cells to inhabit the earth over three billion years ago (Figure 1.1). They are everywhere. They can be found in the air we breathe, in the food we eat, and even within our own body. They are present on earth, which includes humans, animals, plants and other living creatures, soil, water and atmosphere. Microbes can multiply in all the habitats except in the atmosphere. Together, their numbers far exceed all other living cells on this planet.

Microorganisms are relevant to all of us in a multitude of ways. Sometimes, the influence of microorganisms in human life is beneficial whereas at other times it is detrimental. For example, microorganisms are required for the production of bread, cheese, yogurt, alcohol, wine, beer, antibiotics (*e.g.,* penicillin, streptomycin, chloromycetin), vaccines, vitamins, enzymes and many more industrial and food products. Microorganisms are indispensable components of our ecosystem. Fungi and bacteria play an important role in the recycling of organic and inorganic materials through their roles in the carbon, nitrogen and sulphur cycles, thus playing an important part in the maintenance of the stability of the biosphere. They also are a source of nutrients at the base of all ecotropical food chains and webs. In many ways all other forms of life depend on the microorganisms. The use of microbes to reduce or

degrade pollutants, industrial waste and household garbage, a new area referred to as **bioremediation** is being given substantial importance these days. A recent discovery in the 21st century by Professor Jonathan Rhodes with his colleagues at the University of Liverpool, North-West England, says that common edible mushrooms contain a protein *lectin* that can stop cancer cell multiplication. This discovery ultimately could lead to new targets for therapy. *Taxomyces andreanae,* an endophytic fungus known to occur in the USA, is being used to produce **taxol,** an antitumor diterpinoid used in the treatment of some cancers. Taxol was originally obtained from the bark of pacific yew (*Taxus brevifolia*).

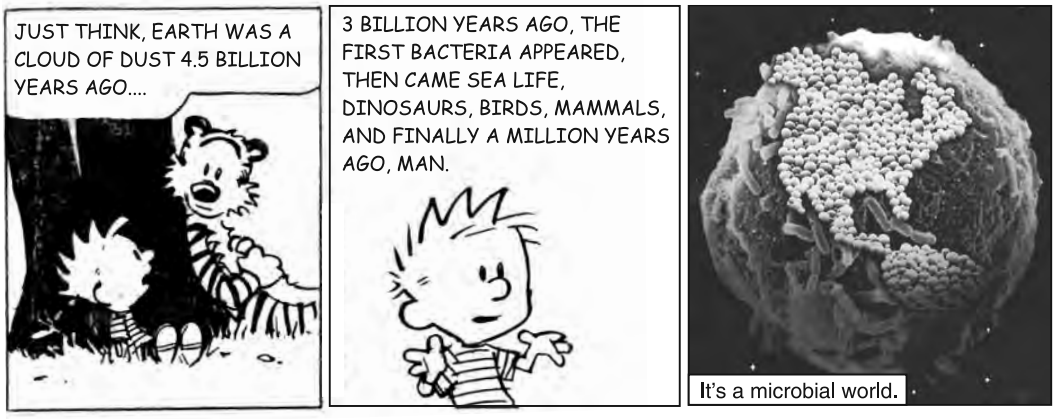


FIGURE 1.1 Evolution of life.

Of course microoganisms also have harmed humans and disrupted societies over the millennia. Microbial diseases undoubtedly played a major role in historical events such as the decline of the Roman empire and conquest of the new world. It was in the year 1347 when **plague** or **'black death'** struck Europe and within 4 years killed 25 million people, that is 1/3 of the population. Over the years, the disease struck again and again, wiping out 75% of the European population by 1431. This dreaded disease is believed to have changed European culture and prepared the way for renaissance.

It was **Robert Koch** (1843–1910), a German bacteriologist, who in 1876 first of all proved that **anthrax** is caused by a microbe *Bacillus anthracis*. Since that time microbiologists have conducted a continuous search for disease causing agents. The past 60 years have witnessed an increase in new and emerging infectious agents such as AIDS virus (HIV, Human Immunodeficiency Virus), SARS, bird's flue, dengue, Eboloa and *Escherichia coli* 0157: H7, and several other older diseases regularly appear to be on the increase. Recently, a correlation has been shown between gastric ulcers and the bacterium (*Helicobactor*) that invades the stomach, between type 1 diabetes and certain coxsackie viruses, and between coronary artery disease and cytomegalovirus infection. In addition to health threat from some microorganisms, many microbes spoil food and deteriorate materials like iron pipes, glass lenses, computer chips, jet fuel, paints, concrete, metal, plastic, paper, and wood pilings.

SCOPE OF MICROBIOLOGY

Microbiology is a specialized area of **biology** (Gr. *bios* = life + *logos* = to study) that **deals with the study of microorganisms.** Roughly speaking, organisms with a diameter of 1 mm or less are microorganisms and fit into the broad domain of microbiology. Microorganisms may be seen only by magnifying their images with the microscope. The study of microorganisms employs certain techniques such as sterilization and use of culture media that are required to isolate and grow them.

Microbiology is one of the largest and most complex of the biological sciences as it deals with many diverse biological disciplines. In addition to studying the natural history of microbes, it also deals with every aspect of microbe—human and environmental interaction. These interactions include: ecology, genetics, metabolism, infection, disease, chemotherapy, immunology, genetic engineering, industry and agriculture. The branches that come under the large and expanding umbrella of microbiology are categorized into pure and applied sciences. **Pure branches** include:

(*i*) ***Bacteriology*:** It is the study of bacteria—the smallest, simplest, prokaryotic, single-celled microorganisms.

(*ii*) ***Mycology*:** It is the study of fungi (achlorophyllous, heterotrophic, eukaryotic, spore-bearing microorganisms with absorptive nutrition and which characteristically have a rigid cell wall containing chitin and/or cellulose). A group of organisms that includes molds, yeasts, mushrooms and puffballs.

(*iii*) ***Protozoology*:** It is the study of protozoans—animal-like and mostly single-celled, eukaryotic organisms.

(*iv*) ***Virology*:** It is concerned with the study of viruses (ultramicroscopic noncellular particles that parasitize living things) and viral diseases.

(*v*) ***Algology or phycology*:** It is the study of algae—simple aquatic organisms ranging from single-celled forms to large sea weeds.

(*vi*) ***Parasitology*:** It is the study of parasitism and parasites—that include pathogenic protozoa, helminth worms and certain insects.

(*vii*) ***Microbial ecology*:** It is the study of interrelationships between microbes and environment.

(*viii*) ***Microbial morphology*:** It is the study of detailed structures of microorganisms.

(*ix*) ***Microbial taxonomy*:** It is concerned with the classification, naming, and identification of microorganisms.

(*x*) ***Microbial physiology*:** It is the study of metabolism of microbes at the cellular and molecular levels.

(*xi*) ***Microbial genetics and molecular biology*:** It is the study of genetic material, structure and function and the biochemical reactions of microbial cells involved in metabolism and growth.

The prominent **applied branches** of microbiology are:

(*i*) ***Industrial microbiology*:** It is concerned with the industrial uses of microbes in the production of alcoholic beverages, vitamins, amino acids, enzymes, antibiotics and other drugs.

(*ii*) ***Agricultural microbiology*:** It is the study of relationships of microbes and crops with an emphasis on control of plant diseases and improvement of yields.

(*iii*) ***Food microbiology*:** It deals with interaction of microorganisms and food in relation to food bioprocessing, food spoilage, foodborne diseases and their prevention.

(*iv*) ***Dairy microbiology*:** It deals with the production of and maintenance in quality control of dairy products.

(*v*) ***Aquatic microbiology*:** It is the study of microorganisms and their activity concerning human and animal health in fresh, estuarine and marine waters.

(*vi*) ***Air microbiology*:** It deals with the role of aerospora in contamination and spoilage of food and dissemination of plant and animal diseases through air.

(*vii*) ***Exomicrobiology*:** It deals with the exploration for microbial life in outer space.

(*viii*) ***Diagnostic microbiology or medical microbiology*:** It deals with the fundamental principles and techniques involved in study of pathogenic organisms as well as their application in the diagnosis of infectious diseases.

(*ix*) ***Immunology* :** It deals with the immune system (*i.e.,* body defenses) that protects against infections and attempts to understand the many phenomena that are responsible for both acquired and innate immunity, in addition to the study of antibody—antigen reactions in the laboratory (*i.e.,* serology).

(*x*) ***Epidemiology and public health microbiology*:** It concerns with monitoring, control and spread of diseases in communities.

(*xi*) ***Biotechnology*:** It is the scientific manipulation of living organisms, especially at the molecular and genetic level to produce useful products.

(*xii*) ***Modern Biotechnology*:** It deals with the construction of microorganisms with specific genetic characteristics by the use of recombinant DNA technology for industrial purposes.

HISTORY OF MICROBIOLOGY—MILESTONES OF PROGRESS

Microorganisms were probably first living things to appear on earth and the study of fossil-remains indicate that microbial infections and epidemic diseases existed thousands of years

ago. It was **Rogen Bacon** (1220–1252) who in the 13th century postulated that disease is produced by invisible living creatures. This suggestion was made again in 1546 by a physician **Girolamo Fracastoro** (1478–1553) of north Italy. Fracastoro wrote a treatise—**De Contagione** in which he said disease was caused by minute "**seed**" or "germ" and was spread from person to person. His work represents a great landmark in the doctrine of infectious diseases and was the result of wide and practical study of infectious diseases prevalent in his days. As early as 1658, a monk named **Athanasius Kircher** (1601–1680) referred to "**Worms**" invisible to the naked eye in decaying bodies, meat, milk, and diarrheal secretions. Although Kircher's description lacked accuracy, he was the first person to recognize the significance of bacteria and other microbes in disease. In 1665, **Robert Hooke**, an English scientist, used a simple lens that magnified objects approximately 30x. He examined thin slices of cork, the bark of oak tree, and found that cork was made of tiny boxes that Hooke referred to as "**Cells**". The work of Hooke was followed by **Matthias Schleiden and Theodore Schwann** who examined a variety of organisms and in 1838–1839 reported that "**all forms of life are composed of cells**", an observation that later became foundation of "**Cell Theory**". The cell theory has since been modified to "**all life is composed of cells that originate from other cells**".

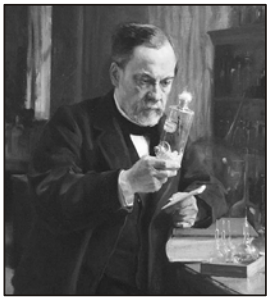
The following section deals with the development of microbiology as a science. The summary of the major events in the historical development of microbiology is presented in Figure 1.2. The discovery of microbiology as a discipline could be traced along the following historical eras.

1. The Discovery Era

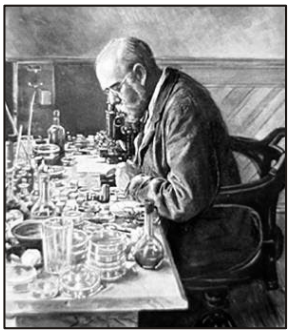
This period concerns with the discovery of microbial world that has been dominated by Antony van Leeuwenhoek. **Antony van Leeuwenhoek** (1632–1723) (Figure 1.3) of Delft, Holland (Netherland) was the first person to observe and accurately describe micoorganisms (bacteria and protozoa) called "**animalcules**" (little animals) in 1673. He also examined blood and other human tissues, including his own tooth scrapping, minerals, and plant materials. Actually, he was a Dutch linen merchant but spent much of his spare time constructing simple microscopes composed of double convex lenses held between two silver plates. He constructed over 250 small powerful microscopes (Figure 1.4) that could magnify around 50 to 300 times and he may have illuminated his liquid specimens by placing them between two pieces of glass and shining light on them at an angle of 45° to the specimen plane. This arrangement would have provided a form of dark-field illumination and made microbes clearly visible. Leeuwenhoek was the first person to produce precise and correct descriptions of bacteria and protozoa using microscope he made himself, because of this extraordinary contribution to microbiology he is considered as the "**father of bacteriology and protozoology**".

GOLDEN AGE OF MICROBIOLOGY (1857–1911)

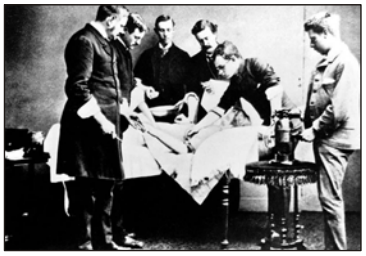
1665	Hooke—First observation of cells
1673	van Leeuwenhoek—First observation of live microorganisms
1735	Linnaeus—Nomenclature for organisms
1798	Jenner—First vaccine
1835	Bassi—Silkworm fungus
1840	Semmelweis—Childbirth fever
1853	de Bary—Fungal plant disease
1857	Pasteur—Fermentation
1861	Pasteur—Disproved spontaneous generation
1864	Pasteur—Pasteurization
1867	Lister—Aseptic surgery
1876	Koch—Germ theory of disease
1879	Neisser—*Neisseria gonorrhoeae*
1881	Koch—Pure cultures
	Finley—Yellow fever
1882	Koch—*Mycobacterium tuberculosis*
	Hesse—Agar (solid) media
1883	Koch—*Vibrio cholerae*
1884	Metchnikoff—Phagocytosis
	Gram—Gram-staining procedure
	Escherich—*Escherichia coli*
1887	Petri—Petri dish
1889	Kitasato—*Clostridium tetani*
1890	von Bering—Diphtheria antitoxin
	Ehrlich—Theory of immunity
1892	Winogradsky—Sulfur cycle
1898	Shiga—*Shigella dysenteriae*
1908	Ehrlich—Syphilis
1910	Chagas—*Trypanosoma cruzi*
1911	Rous—Tumor-causing virus (1966 Nobel Prize)
1929	Fleming—Penicillin
	Griffith—Transformation in bacteria
1934	Lancefield—Streptococcal antigens
1935	Stanley, Northrup, Sumner—Crystallized virus
1941	Beadle and Tatum—Relationship between genes and enzymes
1943	Delbruck and Luria—Viral infection of bacteria
1944	Avery, MacLeod, McCarty—Genetic material is DNA
	Wakman, Schatz—Streptomycin
1946	Lederberg and Tatum—Bacterial conjugation
1953	Watson and Crick—DNA structure
1957	Jacob and Monod—Protein synthesis regulation
1959	Stewart—Viral cause of human cancer
1962	Edelman and Porter—Antibodies
1964	Epstein, Achong, Barr—Epstein-Barr virus as cause of human cancer
1971	Nathans, Smith, Arber—Restriction enzymes (used for recombinant DNA technology)
1972	Berg—Genetic engineering
1975	Dulbecco, Temin, Baltimore—Reverse transcriptase
1978	Woese—Archaea
	Mitchell—Chemiosmotic mechanism
1981	Margulis—Origin of eukaryotic cells
1982	Klug—Structure of tobacco mosaic virus
1983	McClintock—Transposons
1988	Deisenheter, Huber, Michel—Bacterial photosynthesis pigments
1994	Cano—Reported to have cultured 40-million-year-old bacteria
1997	Prusiner—Prions



Louis Pasteur (1822–1895) Demonstrated that life did not arise spontaneously from nonliving matter.



Robert Koch (1843–1910) Established experimental steps for directly linking a specific microbe to a specific disease.



Joseph Lister (1827–1912) Performed surgery under antiseptic conditions using phenol. Proved that microbes caused surgical wound infections.

FIGURE 1.2 Landmark Discoveries in microbiology, highlighting those that occurred during the Golden Age of Microbiology. The period between 1857 and 1911 is called the Golden Age of Microbiology.

FIGURE 1.3 Antony van Leeuwenhoek (1632–1723). First person to observe and describe bacteria and protozoa with his own microscope.

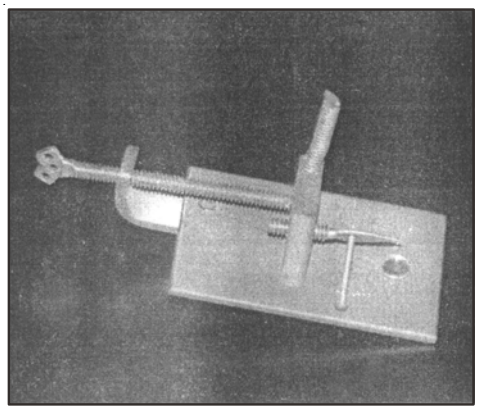


FIGURE 1.4 Replica of van Leeuwenhoek microscope.

2. Transition Period

Spontaneous Generation verus Biogenesis

Though there were a number of significant developments in microbiology during Leeuwenhoek's time and in later years, two noteworthy contributions enhanced the interest in microbes and their relation to diseases: (*i*) The controversy over **spontaneous generation** that said that living organisms could develop from non-living matter (*i.e.* **abiogenesis**); and (*ii*) disease transmission.

Francesco Redi (1626–1697): The ancient belief in spontaneous generation was first of all challenged by Redi, an Italian physician, who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. **Redi, in 1668 put the theory of spontaneous generation to rest** by conducting a simple experiment in which he placed meat in three jars (Figure 1.5). One jar was covered with a fine gauze, second was covered with paper, and the third was left uncovered. Flies entered the jar that was open to air *i.e.,* left uncovered, and landed on meat where they laid their eggs that later developed into maggots. The other two pieces of meat did not produce maggots spontaneously. However, flies were attracted to the gauze covered jars and laid their eggs on the gauze and maggots subsequently developed without access to the meat, indicating that maggots were the off-spring of the flies and did not arise from some "vital source" in the meat as previously believed.

John Needham (1713–1781): He was probably the greatest supporter of the theory of spontaneous generation. In 1749, he proposed that tiny organisms, the animalcules arose spontaneously on his mutton gravy. He had covered the flasks with cork as done by Redi and even heated some flasks. Still the microbes appeared on mutton broth.

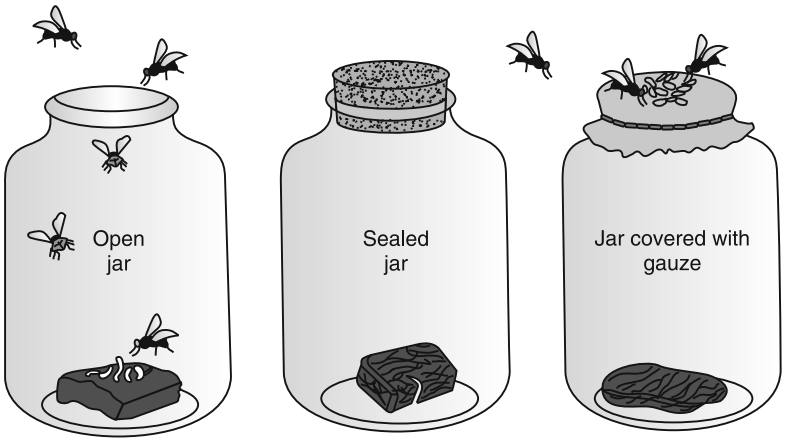


FIGURE 1.5 Redi's experiments refuting the spontaneous generation of maggots in meat. When meat is exposed in an open jar, flies lay their eggs on it, and the eggs hatch into maggots (fly larvae). In a sealed jar, however, no maggots appear. If the jar is covered with gauze, maggots hatch from eggs that the flies lay on top of the gauze, but still no maggots appear in the meat.

Lazzaro Spallanzani (1729–1799): Spallanzani was an Italian naturalist who in 1775 attempted to refute Needham's work by performing extensive experiments. He boiled beef broth for longer period, removed the air from the flask and then sealed the container. Following incubation, no growth was observed by him in these flasks. When he was accused of destroying the vegetative force of the nutrients by over heating, he showed that the heated nutrients could still grow animalcules when exposed to air by simply making a small crack in the neck. Thus, **Spallanzani in 1775 disproved the doctrine of spontaneous generation.**

Franz Schulze (1815–1873) and Theodor Schwann (1810–1882): Needham and other critics countered the doctrine of Spallanzani and stated that **life force** had been killed when the flasks were sealed from oxygen, a gas known to be required in the respiration of animals. This argument was answered 60–70 years later independently by two German scholars-Schulze and Schwann, who were of the view that air was the source of microbes and sought to prove this by passing air through hot glass tubes or strong chemicals into boiled infusions in flasks. The infusion in both the cases remained free from the microbes. But, the die-hard advocates of spontaneous generation were still not convinced and said that the treatment of air by acid or heat had altered the air so that it would not support growth.

Georg Schroeder and Theodor von Dusch: In 1854, Schroeder and von Dusch performed convincing experiments to disprove the theory of spontaneous generation by simply passing air through cotton into flasks containing heated broth. No growth of microbes was observed on the infusions due to the filtering out of microscopic organisms by cotton. Thus, **Schroeder and von Dusch were the first to introduce the idea of using cotton plugs for plugging microbial culture tubes in 1854.**

GOLDEN AGE OF MICROBIOLOGY (1857–1911)

Golden age of microbiology (Figure 1.2) began with the work of Louis Pasteur of France and Robert Koch of Germany who had their own Research Institutes. Here, we find indisputable proof that microbes cause disease. More important, there was an acceptance of their work by the scientific community throughout the world and a willingness to continue and expand the work. During the period, we see the real beginning of microbiology as a discipline of biology.

Louis Pasteur (1822–1895) (Figure 1.2): Pasteur was a French microbiologist, who performed a series of experiments to prove that although microorganisms were present in the air they were not spontaneously produced. To prove that air was the source of microbes, he filled several round–bottomed flasks with nutrient solution and fashioned their openings into elongated swan-neck shaped tubes (Figure 1.6). The flask's opening were freely open to the air but curved so that gravity would cause any airborne dust particle to deposit in the lower part of the neck. The flasks were heated to sterilize the broth and then incubated. No growth occurred even though the contents of the flasks were exposed to the air. Pasteur pointed out that no growth took place because dust and germs had been trapped on the walls of the curved necks but if the necks were broken off so that dust fell directly down into the flask,

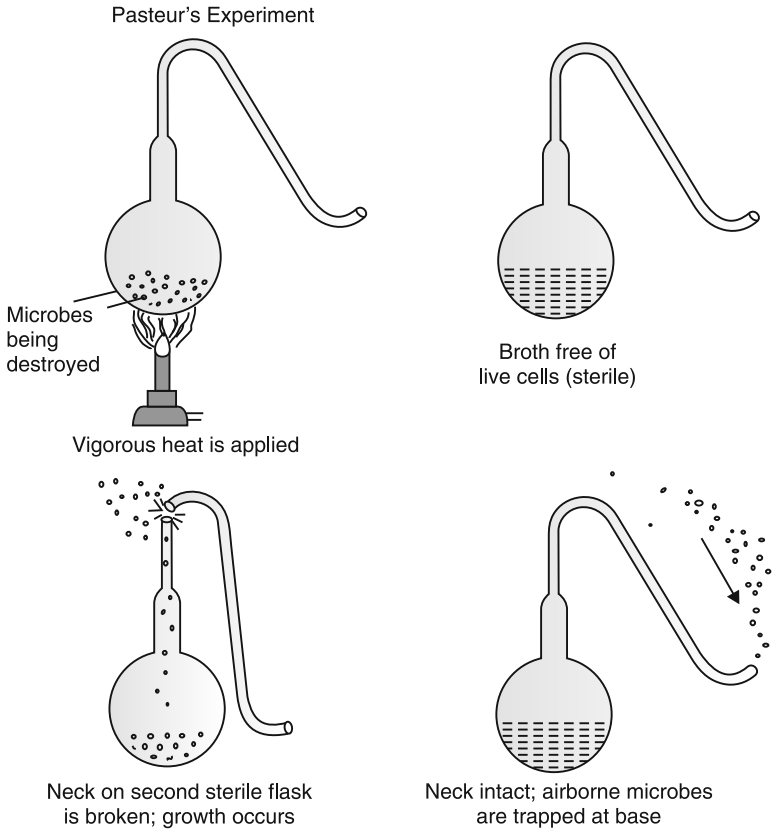


FIGURE 1.6 Swan-neck experiment of Pasteur concerning the spontaneous generation of microbes.

microbial growth commenced immediately. Some of these ingenious little flasks are still on display at the Pasteur Institute in Paris in their original sterile form. This experiment clearly showed that microorganisms present on or in non-living materials such as dust or water were responsible for the contamination of sterile solutions. **Pasteur, thus in 1861 finally resolved the controversy of spontaneous generation versus biogenesis and proved that microorganisms are not spontaneously generated from inanimate matter but arise from other microorganisms.**

John Tyndall (1820–1893), an English physicist, dealt a final blow to spontaneous generation in 1877. Tyndall conducted experiments in an aseptically designed box to prove that dust indeed carried the germs. He demonstrated that if no dust was present, sterile broth remained free of microbial growth for indefinite period even if it was directly exposed to air.

FERMENTATION

Superstition also surrounded the production of wine. The popular belief was that fermentation of grape juice to wine was natural chemical process, involving the breakdown of the protein albumin. Theodor Schwann performed several experiments to disprove the theory of spontaneous generation and was the first person to prove in 1837 that **yeasts were the living things** and determined that the yeast *Saccharomyces cerevisiae* is responsible for alcoholic fermentation.

Louis Pasteur, a Professor of Chemistry at the University of Lille, France was responsible for saving a principal industry of France *i.e.* manufacture of wine and beer. He found that fermentation of fruits and grains, resulting in alcohol, was brought about by microbes and also determined that bacteria were responsible for the spoilage of wine during fermentation. Pasteur in 1867, suggested that mild heating at 62.8°C (145°F) for 30 minutes rather than boiling was enough to destroy the undesirable organisms without ruining the taste of the product, the process was called **pasteurization.** Pasteurization was introduced into the United States on a commercial basis in 1892. Pasteur's work appeared to demonstrate that microbes could be a cause of disease for if they could spoil the wine, perhaps they could also make the body sick. This led to the development of the **germ theory of disease.**

THE GERM THEORY OF DISEASE

Even before Pasteur had proved by experiment that bacteria are the cause of some diseases, many observant students expressed strong arguments for the germ theory of disease. As early as 1546, **Girolamo Fracastoro** wrote a treatise (*De Contagione*) in which he said disease was caused by minute "seed" and was spread from person to person. In 1762, **Von Plenciz**, a Viennese physician, not only stated that living organisms are the cause of disease but suspected that each disease had its own organism. **Agostino Bassi** (1773–1856) demonstrated in 1835 that a silkworm disease was due to a fungal infection. He also suggested that many diseases were due to microbial infections. **M.J. Berkelay** in 1845 proved that the famous famine in Ireland between 1845 and 1847 due to the great potato blight disease which changed the economy of Ireland was caused by a fungus *Phytophthora infestans*. It was

followed by other works: **Johann Schönlein** (1793–1864), a German clinical doctor in 1839, isolated the fungus causing the disease favus. **Oliver Wendell Holmes** (1809–1894) in 1843 reported that puerperal sepsis, a disease of childbirth was contagious and that it was probably caused by a germ carried from one mother to another by midwives and physicians. **Casimir Joseph Davaine** (1812–1882), a French pathologist and parasitologist in 1850 observed *Bacillus* which causes anthrax and transmit the disease by inoculating the infected blood into other animals. The notable human diseases recorded following the germ theory of disease between 1876 and 1912 are summarized in Table 1.1.

TABLE 1.1 **Discovery of the major pathogens following establishment of Germ theory of disease.**

Year of discovery	Scientist	Disease	Causative agent*
1876	Koch	Anthrax	*Bacillus anthracis*
1879	Neisser	Gonorrhea	*Neisseria gonorrhoeae*
1880	Eberth	Typhoid fever	*Salmonella typhi*
1880	Laveran (C. Alphonse)	Malaria	*Plasmodium* spp.
1881	Ogston	Wound infections	*Staphylococcus aureus*
1882	Koch	Tuberculosis	*Mycobacterium tuberculosis*
1882	Loeffler and Schultz	Glandres	*Pseudomonas mallei*
1883	Koch	Cholera	*Vibrio cholerae*
1883–1884	Klebs and Loeffler	Diphtheria	*Corynebacterium diphtheriae*
1885	Loeffler	Swine erysipelas	*Erysipelothrix rhusiopathiae*
1885–1889	Nicolaier and Kitasato	Tetanus	*Clostridium tetani*
1886	Fraenkel	Bacterial pneumonia	*Streptococcus pneumoniae*
1887	Weichselbaum	Meningitis	*Neisseria meningitidis*
1887	Bruce	Undulant fever	*Brucella* spp.
1888	Schütz	Equine strangles	*Streptococcus* spp.
1889	Ducrey	Chancroid	*Hemophilus ducreyi*
1892	Welch and Nuttal	Gas gangrene	*Clostridium perfringens*
1894	Kitasato and Yersin	Plague	*Yersinia pestis*
1895	Moore	Fowl typhoid	*Salmonella gallinarum*
1896	van Ermengem	Botulism (food poisoning)	*Clostridium botulinum*
1897	Bang	Bang's disease (bovine abortion)	*Brucella abortus*
1898	Shiga	Dysentery	*Shigella dysenteriae*
1898	Nocard and Roux	Pleuropneumonia of cattle	*Mycoplasma mycoides*
1905	Schaudin and Hoffmann	Syphilis	*Treponema pallidum*
1906	Bordet and Gengou	Whooping cough	*Bordetella pertussis*
1909	Ricketts	Rocky Mountain spotted fever	*Rickettsia rickettsii*
1912	McCoy and Chapin	Tularemia	*Francisella fularensis*

*Present name; original name in many instances was different.

Lord Joseph Lister (1827–1912) (Figure 1.2), a famous English surgeon is known for his **notable contribution to the antiseptic treatment for the prevention and cure of wound infections.** Lister, impressed with Pasteur's studies on the involvement of microorganisms in fermentation, concluded that wound infections too were due to microorganisms. In 1867, he developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds by the application of phenol on surgical dressings and at times it was sprayed over the surgical areas. He also devised a method to destroy microorganisms in the operation theatre by spraying a fine mist of carbolic acid into the air, thus, producing an antiseptic environment. He also heat sterilized the instruments to be used during surgery. Thus, Joseph Lister was the first to introduce **aseptic techniques** for control of microbes by the use of physical and chemical agents which are still in use today. Because of his notable contributions, Joseph Lister is known as the **Father of antiseptic surgery.**

The first direct demonstration of the role of bacteria in causing disease was provided by **Robert Koch** (Figure 1.2), a German physician who first of all isolated anthrax bacillus (*i.e. Bacillus anthracis,* the cause of anthrax) in 1876. He perfected the technique of isolating bacteria in pure culture. He also introduced the use of solid culture media in 1881 by using gelatin as a solidifying agent. In 1882, he discovered *Mycobacterium tuberculosis.* The most notable contribution of Koch was the establishment of the causal relationship between a microorganism and a specific disease by applying a set of criteria referred to as **Koch's postulates** (Figure 1.7). Koch's postulates were published in 1884 and are the cornerstone of the germ theory of diseases and are still in use today to prove the ***etiology*** (specific cause) of an infectious disease. The postulates are:

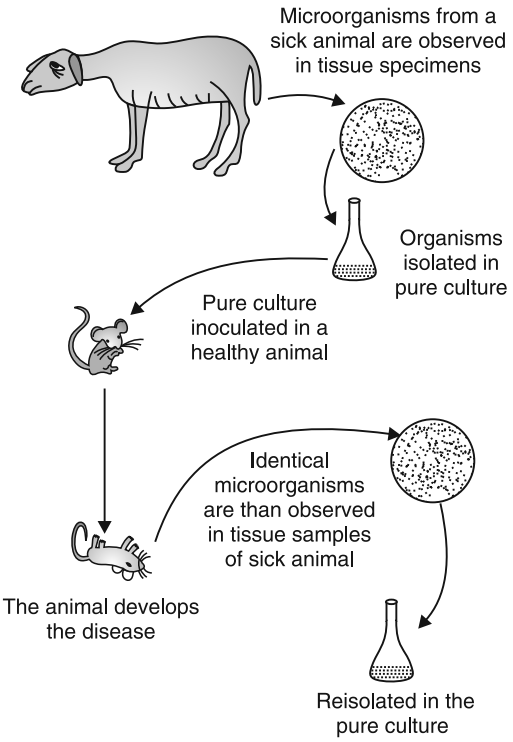


FIGURE 1.7 Koch's postulates.

1. The suspected microorganism must always be found in diseased but never in healthy individuals.
2. The microorganism must be isolated in a pure culture (one free of all other types of microbes) on a nutrient medium.
3. The same disease must result when the isolated microorganism is inoculated into a healthy host.
4. The same organism must by reisolated from the experimentally infected host.

Although viruses and a few other microbes cannot be cultured in artificial media, Koch's postulates are still used today in determining the cause of most infectious diseases.

Robert Koch will be remembered for both his discovery of important disease producing microorganisms and his fundamental contribution to bacteriological techniques.

Gelatin, used by Koch to prepare solid media, was not an ideal solidifying agent because of the two important reasons: (*i*) Since gelatin is a protein, it is digested by many bacteria capable of producing a proteolytic-exoenzyme-gelatinase that hydrolyses the protein to amino acids; and (*ii*) It melts when the temperature rises above 25ºC. **Fanne Eilshemius Hesse (1850–1934)** (Figure 1.8), one of Koch's assistant, first proposed the **use of agar in culture media.** Agar was superior to gelatin because of its higher melting (*i.e.* 96ºC) and solidifying (*i.e.* 40-45ºC) temperature points than gelatin, and was not attacked by most bacteria. Koch's another assistant, **Richard Petri**, in 1887 developed the **Petri dish** (**plate**), a container used for solid culture media. Thus, contributions of Robert Koch, Fannie Hesse and Richard Petri made possible the isolation of pure cultures of microorganisms, and directly stimulated progress in all areas of microbiology especially medical microbiology. A summary of reportable diseases caused by microorganisms and parasites are shown in Table 1.2.

FIGURE 1.8 Angelina and Walther Hesse. The American wife of Koch's assistant suggested solidifying broths with agar as an aid to obtaining pure cultures. She had used it to solidify broths in her kitchen, and we still use it in our labs today.

IMMUNIZATION

The best approach for combating an infectious disease is **prophylaxis,** that is disease prevention. Among the most powerful prophylactic weapons against pathogens are those that enhance people's resistance to disease. During the course of some infectious diseases, the body develops resistance to further attacks by the same pathogen, referred to as **immunity**. Thus a person suffers from such illnesses, *e.g.* measles only once in life. Immunity can be

TABLE 1.2 **Reportable diseases caused by microorganisms and parasites[a]**

Bacterial Diseases	Bacterial Diseases	Bacterial Diseases	Viral Diseases[b]	Algal Diseases
• Anthrax	• Hemolytic uremic syndrome, postdiarrheal	• Streptococcal pneumonia drug-resistant invasive disease	• Hantavirus pulmonary syndrome	None
• Bacterial meningitis				
• Botulism	• Legionnaires' disease		• Hepatitis A, B and C	
• Brucellosis	• Lyme disease	• Streptococcal toxic-shock syndrome	• Hepatitis (unspecified)	**Fungal Disease**
• Chancroid	• Meningitis		• HIV infection, adult	• Coccidiomycosis
• Chlamydial genital infections	• Pertussis (whooping cough)	• Syphilis	• HIV infection, pediatric	
• Cholera	• Plague	• Tetanus	• Influenza	
• Diphtheria	• Psittacosis	• Tuberculosis	• Measles (rubeola)	**Protozoan Disease**
• *Escherichia coli* O157:H7 food poisoning	• Rocky Mountain spotted fever	• Typhoid fever	• Mumps	• Malaria
		• **Viral Diseases[b]**	• Poliomyelitis (polio)	• Cryptosporidiosis
• Gonorrhea	• Salmonellosis	• AIDS (symptomatic cases)	• Rabies (animal and human)	
• *Haemophilus influenzae* infections (invasive)	• Shigellosis	• Arbovirus infection	• Rubella (German measles)	
	• Streptococcal disease, invasive, Group A	• Encephalitis: eastern equine, St. Louis, western equine	• SARS (severe acute respiratory syndrome	
• Hansen's disease (leprosy)				**Helminth Disease**
			• Yellow fever	• Trichinosis

[a]Infectious disease reporting varies by state. This table lists most of the diseases commonly reported to the U.S. Centers for Disease Control and Prevention (CDC).

[b] Although varicella (chickenpox) is not a nationally notifiable disease, the Council of State and Territorial Epidemiologists recommends reporting cases of this disease to the CDC.

induced by introducing harmless living, weakened (attenuated) variants or inactivated bacterial toxins (toxoids) into the body, which consequently develop immune response or resistance without acquiring the disease. This procedure, termed as **vaccination**, was first used successfully in 1798, by **Edward Jenner** (1749-1823), an English physician to prevent smallpox. Jenner was impressed by the observation that countryside milkmaids who contracted **cowpox** (cowpox is a milder disease caused by a virus closely related to small pox) while milking were subsequently immune to smallpox. On May 14, 1796 he proved that inoculating people with pus from cowpox lesions provided protection against small pox. Jenner, in 1798, published his results of 23 successful vaccinators. Eventually, the process was termed as **vaccination**, based on the latin word **'vacca'** meaning cow. Thus the use of cowpox virus to protect small pox disease in humans became popular replacing the risky technique of immunizing with actual smallpox material.

In spite of his success, Jenner never understood the mechanism by which his vaccine provided protection. In 1877, **Louis Pasteur** began experimenting with anthrax vaccine. Pasteur with his Research Associate Emil Roux (1853–1933) while working on chicken cholera, developed the technique of **attenuating** cultures, that are cultures which had lost their ability to cause the disease but gave resistance to the host (*i.e.* immunity). The attenuated culture was called a **vaccine**, in honour of Edward Jenner, who had used cowpox virus to protect humans from smallpox disease. Using this method Pasteur developed anthrax vaccine in 1881. Five years later, Pasteur was successful in preparing vaccine against Rabies. To express gratitude for Pasteur's development of vaccines, an Institute for production of vaccines named after Pateur *i.e.* the Institute Pasteur was established in Pairs, France which remains one of the most important centers of microbiologic work. Louis Pasteur who was a leading microbiologist during the golden age of microbiology (1860–1910) is regarded as a **founding father of Microbiology.**

Elie Metchnikoff (1845–1916) (Figure 1.9) proposed the **phagocytic theory of immunity** in 1883. He discovered that some blood leukocytes, white blood cells (WBCs), protect against disease by engulfing disease-causing bacteria. These cells were called phagocytes and the process, **phagocytosis.** Thus human blood cells also confer immunity, referred to as **cellular immunity**. **Hans Buchner** (1850–1902), a German bacteriologist and immunologist, in 1889, demonstrated the presence of bactericidal substances in blood and in cell-free serum. After the discovery of the causative agent of diphtheria in 1884 by **F. Loeffler,** work was initiated on the production of toxins by microorganisms. **Emile Roux** (1853–1933) and **Alexandre Yersin,** the two notable Franch bacteriologists, demonstrated the production of toxin in filtrates of broth cultures of the diphtheria organism. **Emil von Behring** (1854–1917) and **Shibasaburo Kitasato** (1852–1931), both colleagues of Robert Koch, in 1890, discovered *tetanus (lockjaw) antitoxin*. The causative agent of tetanus (lockjaw), *Tetanus bacillus* (*Clostridium tetani*) had earlier been reported by S. Kitasato in 1889. The production of tetanus antitoxin was demonstrated by injecting inactivated toxin into rabbits, inducing them to produce an antitoxin in blood that would give protection against tetanus. Only a week after the announcement of the discovery of tetanus antitoxin, von Behring in 1890 reported on immunization against diphtheria by diphtheria antitoxin. In 1894 **Roux and J. Martin** began to immunize horses to produce antitoxin against diphtheria. The discovery of the toxin-antitoxin relationship was very important to the development of science of

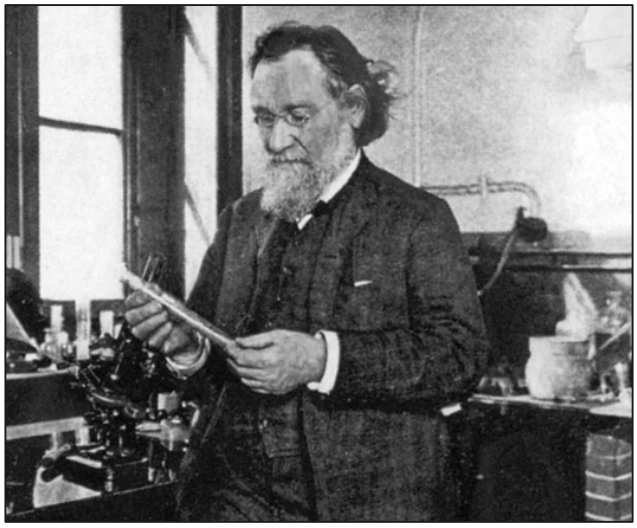


FIGURE 1.9 Elie Metchnikoff (1845–1916). Metchnikoff was one of the first scientists to study the body's defenses against invading microorganisms.

immunology. Discovery of antitoxin work of **Behring** and **Kitasato**, provided evidence that immunity could develop from soluble substances in the blood, now referred to as **antibodies (humoral immunity).**

CHEMOTHERAPY AND WONDER DRUGS

As soon as the relationship between microorganisms and diseases was established by Robert Koch (1876 – Anthrax), Neisser (1879 – Gonorrhea), Eberth (1880–Typhoid fever), Laveran (1880 – Malaria) and several others (Table 1.1), many scientists and physicians intiated work in search of the substances that would kill pathogens without harming the patient. While experimenting with dyes for controlling pathogens, **Paul Ehrlich** (1854–1915) (Figure 1.10) in 1904 found that the dye Trypan Red was active against the trypanosome that causes African sleeping sickness and could be used therapeutically. This dye with antimicrobial activity was referred to as a '**magic bullet**'. Subsequently in 1910, Ehrlich in collaboration with **Sakahiro Hata,** a Japanese physician, introduced the drug **Salvarsan** (arsenobenzol) as a treatment for syphilis caused by *Treponema pallidum*. Ehrlich's work had laid important foundations for

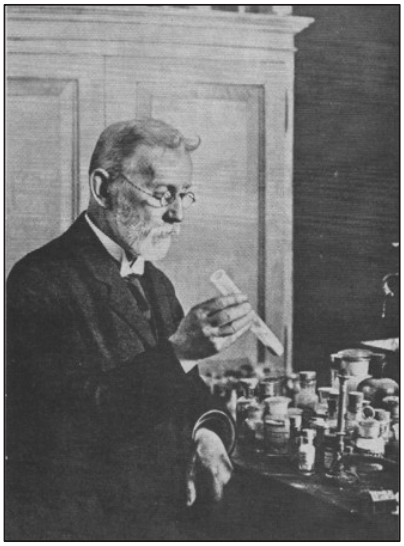


FIGURE 1.10 Paul Ehrlich (1854–1915). Ehrlich was pioneer in the development of chemotherapy for infectious disease.

many of the developments to come and the **use of salvarsan marked the beginning of the era of chemotherapy** (*i.e.* the use of chemicals that selectively inhibit or kill pathogens without causing damage to the patient).

Another breakthrough in chemotherapy that marked the true beginning of broad scale usage of antimicrobial durgs in 1930s was by **Gerhard Domagk** of Germany. Experimenting with numerous synthetic dyes, Domagk in 1935 reported that **Prontosil** a red dye used for staining leather, was active against pathogenic streptococci and staphylococci in mice even though it had no effect against that same infectious agent in a test tube. Shortly after, in the same year *i.e.* 1935, two French scientists **Jacques** and **Therese Trefonel** showed that the compound Prontosil was broken down within the body of the animal to **salfanilamide** (sulfa drug) the true active factor. **Colebrook** and **Kenny** confirmed the clinical effectiveness of suflanilamide in puerperal fever, a streptococcal infection. **Domagk was awarded Nobel prize in 1939 for the discovery of the first sulpha drug.**

The credit for the discovery of first **'*wonder drug*'**, penicillin in 1929 goes to **Sir Alexander Fleming** (Figure 1.11) of England, a Scottish physician and bacteriologist. The discovery of penicillin is a fascinating and fortunate accident. Fleming had been actually interested in searching something that would kill pathogens ever since working on wound infections during the First World War (1914–1918). One day in September, 1928 upon his return from a week's vacation, Fleming observed that a plate of *Staphylococcus aureus* had become contaminated with a green mold *Penicillium notatum* which had accidentally fallen in the plate. Observing this plate, Fleming noted that the colonies of *Staphylococcus* bacterium were evidently being destroyed by the nearby *Penicillium* colonies (Figure 1.12). Rather than discarding the contaminated plate, he speculated that the mold was producing a diffusible substance that inhibited the bacterial growth. Fleming isolated and subcultured the mold for further study. He extracted from the fungus a compound which he called **penicillin,** after the name of the producer organism *Penicillium notatum,* that could destroy several pathogenic

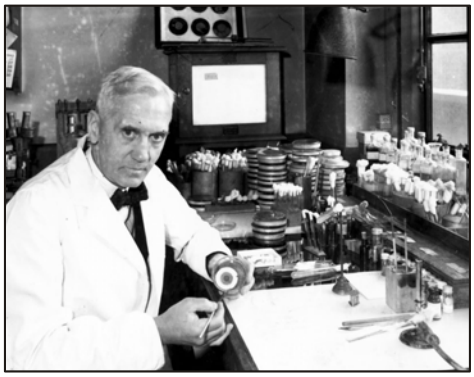


FIGURE 1.11 Alexander Fleming. Fleming discovered the antibacterial properties of penicillin.

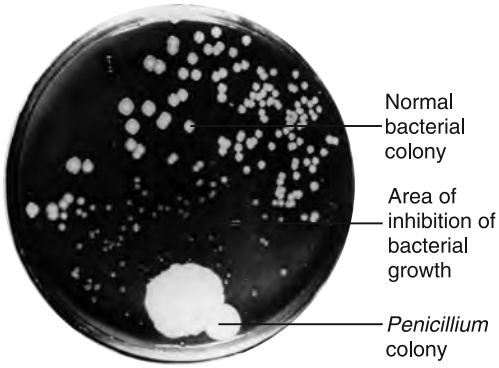


FIGURE 1.12 The discovery of penicillin. Alexander Fleming took this photograph in 1928. The colony of the mold *Penicillium notatum* accidentally contaminated the plate and inhibited nearby bacterial growth.

bacteria. Thus, Sir Alexander Fleming in 1929 discovered the first **antibiotic** (Gr. *anti* = against + *bios* = life, the microbial products that can kill susceptible microorganisms and inhibit their growth) penicillin.

Fleming's monumental work went practically unnoticed until 1940 when **Sir Howard W. Florey**, and **E.B. Chain**, at Oxford University, developed methods for industrial productions of penicillin in England. Not only they determined its value as a therapeutic agent during clinical trials conducted in 1941, they were also responsible for placing it before the American Medical World and for gaining the interest of American industry. The commercial production of penicillin in the USA began in 1941. **Fleming, Florey and Chain shared the Medicine Nobel Prize in 1945 for the discovery and production of penicillin.** *Penicillium notatum* has been replaced with *Penicillium chrysogenum* for the commercial production of penicillin and the current strain of this species modified through mutation, yields 85,000 units/ml of the medium (the original mold *P. chrysogenum* synthesized 60 units/ml).

During World War II, the demand for chemotherapeutic agents for treating wound infections led to the development of a production process for penicillin and the beginning of the era of antibiotic research. **S.A. Waksman** at the Rutgers University, USA discovered another antibiotic, **streptomycin,** produced by two strains of actionomycete *Streptomyces griseus* in 1944. This antibiotic was the result of patient screening of nearly 10,000 cultures isolated from soils, composts and other natural substances that harbour large numbers of organisms capable of producing antibiotic substances. **Waksman received the Medicine Nobel prize in 1952 for his discovery of streptomycin used in the treatment of tuberculosis,** a bacterial disease caused by *Mycobacterium tuberculosis*, that had been discovered by Robert Koch in 1882.

By 1950, three other microorganisms were identified that produced antibiotics, such as **chloramphenicol** (chloromycetin) from *Streptomyces venezuelae* by **Dr. Paul R. Burkholder** in 1947; **aureomycin** from *Streptomyces aureofaciens* by **Dr. B.M. Dugger** in 1948; and **terramycin** from *Streptomyces rimosus* by **Finlay, Hobby and collaborators** in 1950.

Antibiotic production continues to be the most important area of industrial microbiology. Intensive screening programmes in all industrial countries continue to increase the number of described antibiotics–513 antibiotics were known in 1961, 4076 in 1972, 7650 in 1985, and currently there are over 8,000 antibiotics known. Of these only a few are being used as chemotherapeutic agents. Most of the clinically important antibiotics were discovered between 1929 and 1963 (Table 1.3). The most effective agents were isolated from the molds (*Penicillium* and *Cephalosporium*) and from members of the bacterial genera *Streptomyces* and *Bacillus*. All these organisms are common contaminants in the soil and air.

There has been a steady launched of antibiotics since 2000, averaging three approvals every two years (*i.e.*, 30 antibiotics between 2000 and 2015 worldwide) with a notable spike of seven approvals in 2014. There is a growing global recognition that the continued emergence of multidrug resistant bacterial poses a serious threat to human health. Action plans released in 2016 by the World Health Organization (WHO) and the governments of UK and the USA in particular regonised that discovering new antibiotics, particularly those with new modes of action, is one essential elements to avert future catastrophic pandemics.

MICROBIOLOGY IN THE 20TH CENTURY: ERA OF MOLECULAR BIOLOGY

With the recognition of the unity of the biochemical life processes in microorganisms and higher forms of life, including human beings, the use of microorganisms as a tool to explore fundamental life processes became attractive due to the following facts:

- They reproduce (grow) very rapidly;
- Can be cultured (grown) in small and vast quantities conveniently and rapidly;
- Their growth can be manipulated easily by physical and chemical means;

TABLE. 1.3 **Major antibiotics and drugs discovered between 1929 and 1963**

Antibiotic	Year of Discovery	Source	Antimicrobial spectrum
Penicillin G	1929	*Penicillium notatum*	Gram-positive bacteria and Neisseriae
Sulfa drugs*	1935	Chemical synthesis	Broad spectrum
Griseofulvin	1939	*Penicillium griseofulvum*	Fungi (dermatophytes and plant pathogens)
Streptomycin	1944	*Streptomyces griseus*	Gram–negative bacteria and Mycobacteria
Bacitracin	1945	*Bacillus subtilis*	Gram-positive bacteria
Chloramphenicol	1947	*Streptomyces venezuelae*	Gram-positive and Gram-negative bacteria
Polymyxin	1947	*Bacillus polymyxa*	Gram-negative bacteria
Aureomycin (Chlorotetracycline)	1948	*Strepotomyces aureofaciens*	Gram-positive and gram-negative bacteria
Cephalosporin	1948	*Cephalosporium* sp.	Gram-positive bacteria
Neomycin	1949	*Streptomyces fradiae*	Gram-negative bacteria
Nystatin	1950	*Streptomyces noursei*	Fungi
Tarramycin (Oxytetracycline)	1950	*Streptomyces rimosus*	Broad spectrum
Erythromycin	1952	*Streptomyces erythraeus*	Gram-positive bacteria
Cycloserine	1954	*Streptomyces* sp.	Mycobacteria (especially *M. tuberculosis*
Amphotericin B	1956	*Streptomyces nodosus*	Fungi
Vancomycin	1956	*Streptomyces orientalis*	Gram-positive bacteria
Metronidazole*	1957	Chemical synthesis	Protozoa, anaerobic bacteria
Kanamycin	1957	*Streptomyces kanamyceticus*	Gram-negative bacteria
Rifamycin	1957	*Nocardia mediterranei*	Gram-positive bacteria and Mycobacteria
Gentamicin	1963	*Micromonospora purpurea*	Gram-negative bacteria

*Sulfa drugs and Metronidazole are not antibiotics since these are not produced by microorganisms.

- Their cells can be broken apart or the contents can be separated into fractions of various particle sizes. Because of these characteristics microorganisms were used as research models to determine exactly how various life processes take place in terms of specific reactions and the specific structures involved.

During the 1940s, a closer relationship was established between microbiologists, physicists, genetists, biochemists and biologists resulting in the creation of a new discipline what is now called **molecular biology.** It can be defined as the program of interpreting the specific structures and function of organisms in terms of molecular structures. **George W. Beadle** and **Edward L. Tatum** both US scientists, were the pioneers in the area of microbial genetics. They studied the relationship between genes and enzymes in 1941 using mutants of the bread mold fungus *Neurospora crassa* and gave the concept of **one-gene-one-enzyme** hypothesis. Using mutants of *Neurospora,* they demonstrated that there was a direct relationship between a single gene and a single enzyme. Beadle and Tatum hypothesized that the synthesis of the compounds essential for cell growth must be under genetic control. To prove it experimentally, they irradiated asexual spores (called conidia) of wildtype *Neurospora* that can synthesize all metabolites (such as amino acids, purines, pyramidines and vitamins) from the basic ingredients added in the medium, to X-rays or UV light, which will induce mutations affecting specific steps in metabolism (Figure 1.13). Beadle and Tatum took irradiated spores of *N. crassa* and crossed them to wild type spores of the opposite mating type, since the fungus is heterothallic. Some yielded progeny that could not grow in a minimal medium but could grow on an enriched medium. However, if the mutants were grown on a minimal medium supplemented with one or more amino acids, purines, pyrimidines and vitamins, some mutants grow. These growth requirements were inherited thereby suggesting that *Neurospora* had a defective gene for a metabolic step in the synthesis of the factor. They showed that each mutation resulted in a requirement for one growth component. Further experiment conducted on the biochemical steps of the pathways for the synthesis of individual growth components identified which enzyme was defective in each mutant. Beadle and Tatum concluded that a defect in one gene produced a single defect in an essential enzyme resulting in the growth factor requirement, that is one-gene one-enzyme. **Lederberg, Beadle and Tatum were awarded the Nobel prize in 1958 for the discovery of "one-gene–one-enzyme" hypothesis.**

Max Delbrück and **Salvadore Luria** in 1943 described the genetic nature of viruses. They also proved that gene mutations were truly spontaneous and not directed by the environment. DNA is the genetic material and carried genetic information during transformation in bacteria was demonstrated in 1944 by **Oswald T. Avery, Colin M. MacLeod and Maclyn McCarty. Joshua Lederberg**, an American, who shared Nobel Prize with Beadle and Tatum for "one-gene-one-enzyme" hypothesis is considered to be the most famous microbial genetists of the 20th century for his landmark discoveries in bacterial genetics, bacterial conjugation and transduction.

In 1952, **Joshua Lederberg** first of all introduced the term **'plasmid'** to describe nonchromosomal genetic material in bacteria. In collaboration with **Norton Zinder,** a student in his laboratory at Wisconsin, USA, Lederberg discovered that genetic information could be transferred between bacteria by bacteriophage, that process was known as **transduction**. At

the University of Wisconsin, Lederberg along with his wife **Esther**, developed a unique method of studying bacterial mutants, now known as **'replica plating'**. Using this method it is possible to transfer bacterial colonies form one agar growth plate to other so that each

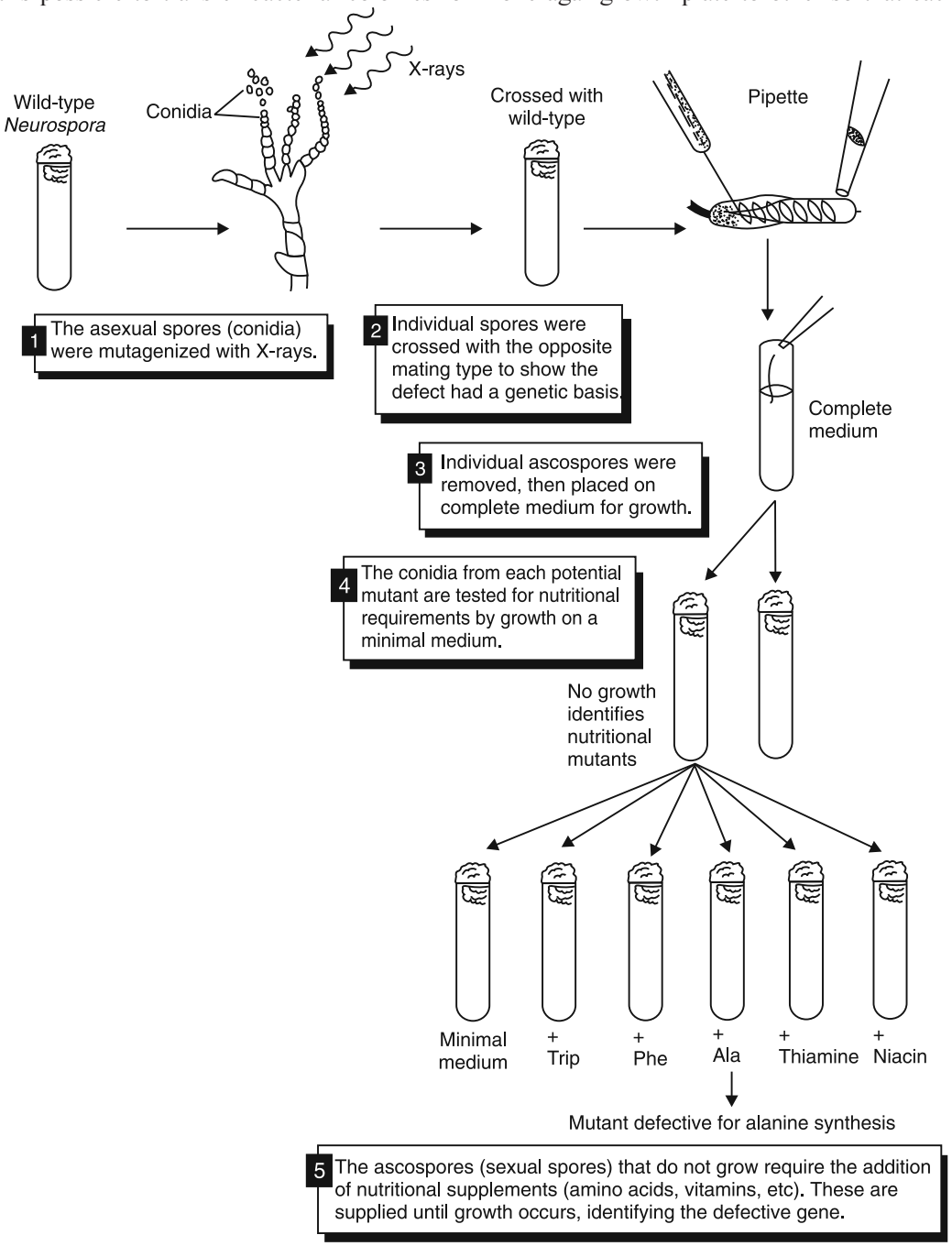


FIGURE 1.13 Beadle and Tatum experiment on *Neurospora crassa* to prove the concept of "one-gene one-enzyme" hypothesis.

new plate is an exact replica of the original. In this way, it is possible to detect and isolate rare mutants with differing nutritional requirements. With this technique, Lederberg showed that **mutations in bacteria occur randomly and spontaneously**, thus confirming a long held hypothesis in the field of evolutionary genetics. Thus, discoveries made by Lederberg in bacterial genetics—transduction and conjugation in bacteria have made the science of bacterial genetics and have subsequently spawned many advances, including aspects of modern molecular genetics of gene cloning. It can be said that **Joshua Lederberg** single-handedly changed the nature of bacterial genetics and changed the course of both genetics and biochemistry.

Watson and **Crick** in 1953, made most rememberable discovery in genetics by discovering the molecular structure of DNA, providing framework for understanding molecular basis of inheritance and expression of genetic information. The significance of these discoveries in molecular biology to biology is understood by the fact that about 1/3 of the Noble Prizes have been awarded to researchers for their work in the area of microbiology (Table 1.4).

NOBEL PRIZE AWARDS FOR RESEARCH INVOLVING MICROBIOLOGY (1901–2022)

Since 1900, Nobel Prizes named after **Alfred Nobel**, have been awarded annually to outstanding scientists in Physiology or Medicine for discoveries that led to benefit for human beings, by Nobel Assembly at Karolinska Institute, Stockholm, Sweden. Microbiology has been in the forefront of research in medicine and biology as revealed by Table 1.4. **Emil A. von Behring** of Germany was the first to receive Nobel Prize in 1901 for work on development of vaccines against diphtheria. Nobel prize awards for research involving microbiology, from 1901 through 2022 are listed in Table 1.4.

TABLE 1.4 **Scientists awarded Nobel Prizes for their contributions involving microbiology (1901–2022)**

Year of award	Contribution	Nobel Laureates	Country of Birth
1901	Diphtheria antitoxin	Emile A. von Behring	Germany
1902	Cause of malaria	R. Ross	Great Britain
1905	Tuberculosis research	R. Koch	Germany
1907	Role of protozoa in disease	C. Laveran	France
1908	Work on immunity	P. Ehrlich E. Metchnikoff	Germany Russia
1913	Work on anaphylaxis	C. Richet	France
1919	Discoveries about immunity	J. Bordet	Belgium
1928	Work on typhus fever	C. Nicolle	France
1930	Discovery of human blood groups	K. Landsteiner	United States
1939	Antibacterial effect of prontosil	G. Domagk	Germany
1945	Discovery of penicillin and its therapeutic value	A. Fleming E.B. Chain H.W. Florey	Great Britain Great Britain Australia

(Contd.)

1951	Development of yellow fever vaccine	M. Theiler	South Africa
1952	Discovery of streptomycin	S.A. Waksman	United States
1954	Cultivation of poliovirus in tissue culture	J.F. Enders	United States
		T.H. Weller	United States
		F. Robbins	United States
1957	Discovery of the first antihistamine	D. Bovet	Italy
1958	Microbial genetics	G.W. Beadle	United States
		E.L. Tatum	United States
		J.Lederberg	United States
1959	Discovery of enzymes catalyzing nucleic acid synthesis	S. Ochoa	United States
		A.Kornberg	United States
1960	Discovery of acquired immune tolerance to tissue transplants	F.M. Burnet	Australia
		P.B. Medawar	Great Britain
1962	Discoveries concerning the structure of DNA	F.H.C. Crick	Great Britain
		J.D. Watson	United States
		M. Wilkins	Great Britain
1965	Discoveries about the regulation of genes	F. Jacob	France
		A. Lwoff	France
		J. Monod	France
1966	Discovery of cancer viruses	F.P. Rous	United States
1968	Deciphering of the genetic code	R.W. Holley	United States
		H.G. Khorana	United States
		M.W. Nirenberg	United States
1969	Discoveries concerning viruses and viral infection of cells	M.Delbrück	United States
		A.D. Hershey	United States
		S.E. Luria	United States
1972	Research on the structure of antibodies	G.Edelman	United States
		R. Porter	Great Britain
1975	Discovery of RNA-dependent DNA synthesis by RNA tumor viruses; reproduction of DNA tumor viruses	H. Temin	United States
		D. Baltimore	United States
		R. Dulbecco	United States
1976	Discoveries concerning new mechanism for the origin and dissemination of infections diseases (especially Kuru)	B.S. Blumberg	United States
		D.C. Gajdusek	United States
1977	Development of the radioimmunoassay technique	R. Yalow	United States
1978	Discovery of restriction enzymes and their application to the problems of molecular genetics	H.O. Smith	United States
		D. Nathans	United States
		W. Arber	Switzerland
1980	Discovery of the histocompatibility antigens	B. Benacerraf	United States
		G. Snell	United States
		J. Dausset	France
	Development of recombinant DNA technology (Berg); development of DNA sequencing techniques (Chemistry Prize)	P. Berg	United States
		W. Gilbert &	Unites States
		F. Sanger	Great Britain
1982	Development of Crystallographic electron microscopy and the elucidation of the structure of viruses and other nucleic acid protein complexes (Chemistry Prize)	A. Klug	Great Britain
1984	Development of the technique for formation of monoclonal antibodies (Milstein & Kohler); theoretical work in immunology (Jerne)	C. Milstein	Great Britain
		G.J. F. Kohler	Germany
		N.K. Jerne	Denmark

(Contd.)

1986	Development of the transmission electron microscope (Physics Prize)	E. Ruska	Germany
1987	The genetic principle for generation of antibody diversity	S.Tonegawa	Japan
1988	Crystallization and study of the photosynthetic reaction center from a bacterial membrane	J.Deisenhofer, R. Huber and H. Michel	Germany
	Development of drugs for the treatment of cancer, malaria and viral infections	G. Elion G. Hitchings	United States United States
1989	Discovery of oncogenes	J.M. Bishop H.E. Varmus	United States United States
	Discovery of catalytic RNA	S. Altman T.R. Cech	United States United States
1993	Invention of the polymerase chain reaction Development of site-directed mutagenesis Discovery of split genes (genes can be discontinuous)	K.B. Mullis M. Smith R.J. Roberts & P.A. Sharp	United States United States United States United States
1996	Discovered how cytotoxic T cells recognize virus-infected cells prior to destroying them	Peter C. Doherty and Rolf M. Zinkernagel	Australia Switzerland
2003	Discovered water and ion channels in plasma membranes	Peter Agre and Roderick MacKirron	United States
2004	Discovered how cells dispose off unwanted proteins in Proteasomes	Aaron Ciechanover, Avram Hershko, and Irwin Rose	Israel Israel United States
2005	Discovered that *Helicobacter pylori* causes peptic ulcers	Barry Marshall and J. Robin Warren	Australia
2006	Discovered RNA interference (RNAi), or gene silencing, by double-stranded RNA	Andrew Fire and Craig Mello	United States
2008	Discovery of human papilloma viruses causing cervical cancer	Harald zur Hausen	Germany
2008	Discovery of human immunodeficiency virus (HIV) causing AIDS	Francoise Barré-Sinoussi and Luc Montagnier	France
2010	Detailed study of the structure and function of ribosomes	Venkatraman Ramakrishnan, Thomas A. Steitz, and Ada E. Yonath	India United States Israel
2011	Activation of innate immunity	Bruce A. Beutler and Jules A. Hoffman	United States
2015	Therapy against infections caused by roundworm parasites Novel therapy against malaria	William C. Campbell & Satioshi Omura Youyou Tu	United States Japan China
2016	Mechanisms for autophagy (self-eating yeast)	Yoshinori Ohsumi	Japan
2018	Phase display of peptides and antibodies	George P.Smith Sir Gregory P.Winter	USA UK
2020	Discovery of hepatitis C virus	Harvey J.Alter Michael Houghton Charles M.Rice	USA UK USA
2020	For the development of a method for genome editing	Emmanuelle Charpentier Jennifer A.Douchna	France USA

TAXONOMY AND NOMENCLATURE OF LIVING ORGANISMS

An incredible diversity of life is present on our planet with new species of all types being constantly discovered. The formal system of organizing, classifying and naming living things is called **taxonomy** (Gr. *taxis* = arrangement + *nomos* = name). In other words, the primary goals of taxonomy are classification, nomenclature and identification. These three areas are interrelated and play a vital role in keeping a dynamic inventory of the extensive array of living things. Once the characteristics of microorganisms have been determined and appropriately catalogued the process of classification begins. The orderly arrangement of organisms into groups preferably in a format that shows evolutionary relationships is termed as **classification. Nomenclature** is the process of assigning names to the various taxonomic ranking of each microbial species. The process of discovering and recording the distinguishing features of organisms is called **identification.**

Aristotle (4th century B.C.) was probably the first to group all organisms and categorized them as either plants or animals. In 1753, **Carolus Linnaeus,** a Swedish botanist, laid down the basic rules for taxonomic categories or taxa and gave the **binomial system of nomenclature** *i.e.,* **naming of an organism by two names—genus** and **species.** The name of the organism starts with the generic (Genus) name that is always capitalized, which is followed by the **species** name that begins with a lowercase (small letter). Both should be written in italics (or underlined if italics are not available), as follows: ***Corynebacterium diphtheriae.*** The source of nomenclature is usually Latin or Greek. The name of species is merely a convenient label. A number of species have been named in honour of a scientist who originally discovered the microbe or who has made outstanding contribution to the field. For example *Escherichia coli*, the generic name of organism named after **Theodor Escherich**, a German bacteriologist who first described the bacterium, and specific name, *coli* refers to the colon and is appropriate because this organism is an enteric resident of humans. Other names may designate a characteristic of the microbe (shape, colour) *e.g., Proteus vulgaris* is Latin for "common organisms of many shapes", a location where it is found, a place from where it was recorded or a disease it causes. The Linneaus system provides each organism with a unique name and arranges them into groups called **taxa** (categories) with other similar organisms to reflect their phylogeny and evolutionary relatedness. The main taxa or groups in a classification scheme are organised in several descending ranks beginning with the **Kingdom** the largest and followed by **Phylum** or **Division, Class, Order, Family, Genus and Species.**

The classification of microorganisms began in 1674 with the invention of light microscope and today is a discipline based on increasingly complex criteria. The first phylogenetic trees of life were constructed on the basis of just two Kingdoms: **Plantae** and **Animalia.** In 1866, a German Naturalist **Ernest Haekel** proposed that the bacteria, algae, fungi and protozoa that lacked tissue differentiation be removed from the plant and animal kingdoms and be separated in a third kingdom **Protista.**

With the introduction of microscopy, it was discovered by 1940 that in some organisms *e.g.,* typical bacteria, the genetic material was not enclosed by a nuclear membrane (Figure 1.14). This remarkable discovery, the absence of nuclear membrane bound internal structure

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